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WATER DEFICIT AND THE ACTION OF VITAMINES, AMINO-COMPOUNDS, AND SALTS ON HYDRATION¹

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It is well known to any one who has seriously examined cells in a living condition that protoplasm is a viscous substance which at different times or in different parts of the same protoplast may vary in consistency from a liquid to that of a firm jelly, a behavior characteristic of an emulsoid colloid. This term in the present instance is applied to substances which in a condition of hydration exist in two distinct phases in the mass; in one, a few or many molecules of the solid substance are combined or held together by adsorption with a relatively small number of water molecules to form a denser phase, which in the more liquid condition of the mass floats in or is surrounded by a more fluid phase in which the solid particles sustain a much smaller proportion to the water.

If we begin to form our picture of the colloid in this condition, which would be that of melted agar or gelatine, we shall be ready to follow its transformation to that of a jelly by visualizing an action by which the surface tension of the aggregates of molecules is increased by lowered temperatures or other causes, the aggregates coming together to form mesh-works or honeycombs, running through or partially enclosing the material of the more liquid phase. The possibilities implied in the reversal of these phases, important as they may be, may be disregarded during the present discussion.

Living matter is anything but such a simple substance. The results of all of our examinations by physical and chemical methods are to the effect that the living matter of plants includes the following groups of substances which may assume the colloidal conditions described above: First, the nitrogenous substances, which include not only albumin and all its derivatives, but also synthetic compounds. Next in importance, and constituting perhaps the greater part of the mass, are the pentosans or mucilaginous sugars which may be formed in carbohydrate metabolism in any part of the mass when hexoses are converted into pentoses and these are condensed into the pentosans by dehydration. The presence of such fatty acids as stearic, palmitic, and oleic, and the readiness with which they form soaps by combination with potassium, sodium, and magnesium, make it also certain that such substances are an invariable component of the biocolloids.

¹ Invitation paper read before the Physiological Section of the Botanical Society of America, in the symposium on biophysics, at Chicago, December 28, 1920.

In addition, a certain amount of lipins may be present, but as their possible hydration action in the colloidal mass must be very slight, they may be left for consideration in a discussion of other phases of protoplasmic action.

When the general properties of these main components of living matter are reviewed, it is seen that the albuminous or proteinaceous substances are amphoteric, behaving as either acids or bases according to the hydrogen-ion concentration of the solution, that they generally unite with the highest proportion of water when in an acidified condition, and that their hydration is also facilitated in lesser degree by the action of bases and their salts. The pentosans or mucilages are weak acids and undergo the highest degree of hydration in neutral or slightly alkaline solutions, in extremely dilute solutions of the common salts of potassium, magnesium, calcium, and sodium, or in the presence of certain amino-compounds described in previous papers. That the nitrates, chlorides, and sulphates of these metals may also increase the hydration capacity of the pentosans will be demonstrated in the present paper.

The soaps are characterized by their capacity for forming films, and their high hydration capacity is altered to such degree by variations in the hydrogen-ion concentration of the solutions as to make them "sensitizers" as to acidity in any colloidal mass into which they may enter. It is to be noted that the range of acidity or alkalinity of interest to the physiologist in the present connection is that which lies between the measurements expressed by the symbols $\text{PH} = 3$ and $\text{PH} = 11$, and that of the salts as chiefly between 0.001 and 0.0001 M.

Taking into consideration the complex conditions suggested above, it is reasonable to infer, since we have as yet devised no means of direct observation, that, as the more complex proteins and the pentosans do not dissolve or diffuse in each other, they form separate aggregates, and that the sponge or meshwork of the solidified biocolloid or protoplasm must therefore consist of an interwoven meshwork of the two. This inference may not be carried safely beyond a certain point, however, nor to imply that no nitrogenous substances of the cell may engage, or be adsorbed by, the pentosans, since the results of the action of histidine, glycocoll, alanine, asparagine, and phenylalanine upon agar go far to suggest the possibility of such unions. The soaps which may be present in the cell colloids are not known to form combinations with the carbohydrates, and, although some uncertainty exists as to what disturbances may be caused by their contacts with the proteins, yet it may be assumed for the present that they form films enclosing the more solid phase of the double meshwork, being thus an interphase in the colloidal machine, highly sensitive to the action of the hydrogen-ion and adding greatly to the complexity of the possible action of the mass as a whole in response to various solutions and environmental conditions.

In this my results are in accord with those of Dr. Clowes, who ascribes

variations in permeability to effects of electrolytes and metabolic products on interfacial soap films.²

Referring to the picture of the colloidal mechanism described above, it is obvious that, while the proteins or albumins and the pentosans must form separate strands or plates, the smaller aggregates in the more liquid portion or phase mingle with each other, and it might also be suggested that various unions might take place between molecular groups of pentosans with amino-compounds or salts, and that actual salts might be formed by the albumins when the colloid is immersed in hydroxides or salts such as those of potassium, sodium, or magnesium. Here then we have a crude statement of a theory of the colloidal condition of protoplasm upon which extensive experimentation as to the action of bases, salts of common metals, acids, amino-compounds, and vitamins has been carried on with the acquisition of results of positive value. These reactions are our real aim, and after we have crossed our bridge of hypothesis to the solid ground of facts, the fate of the bridge which may have served us well is of minor importance.

The arrangement of living matter inferred has much to support it. It is one in which the separate components of the colloidal machine each present a separate and individualized capacity for hydration changes under any set of conditions or at any given temperature for example, and when immersed in water would move with differentiated speed to saturation or satisfaction.

If the water in which the colloid is immersed holds substances in solution the ions of which may form combinations with the main components mentioned above, their capacity for holding water in combination may be altered, and combinations or splittings in the metabolism of substances in protoplasmic colloids may also exert such action. Thus, a dissociation resulting in the freeing of hydrogen ions tends to increase the hydration capacity of the protein strands or aggregates of the mass might lessen that of the pentosans and soaps. Slightly acid amino-compounds as glycocoll would increase the hydration of the pentosans while exerting practically no effect on the albumin or albumin derivatives. It is evident without further elaboration that in the albumin-pentosan-soap machine we have a mechanism capable of an almost endlessly diversified action in swelling and growth.

With so much prelude we may now advantageously turn to a consideration of recently acquired results obtained by testing the action of salts, balanced solutions, amino-compounds, and vitamins on such colloidal mechanisms and on biocolloids and cell masses, living and dead.

In an earlier paper³ I had advanced the idea that the common metals

² Clowes, G. H. A. On the action exerted by antagonistic electrolytes on the electrical resistance and permeability of emulsoid membranes. *Proc. Soc. Exper. Biol. and Med.* 15: 108.. 1918.

³ MacDougal, D. T. Growth in organisms. *Science*, 49: 599-605. 1919. (See p. 11 of reprint).

which enter into nutritive solutions, as potassium, magnesium, sodium, and calcium, might find their chief importance in restricting, limiting, or defining hydration. Such an action is exerted by these bases in the form of hydroxides when tested at 0.01 N. MacDougal and Spoehr⁴ found later that the hydroxides of the strong metallic bases limit the hydration of agar according to their position in the electromotive series, the least swelling taking place under the action of the strongest base at concentrations of 0.01 N, with the apparent exception of rubidium. Beginning with the strongest, the series runs $K > (Rb) > Na > Li$. The various effects of barium, calcium, and strontium are not so clearly determined, and the quantitative relations of these metals are not known definitely. Hydration values of agar at 0.01 N were $Sr(OH)_2 = 815$, $Ca(OH)_2 = 860$, $Ba(OH)_2 = 900$.

These concentrations are far beyond the actual range of conditions in the cell, however, and when reduced concentrations were used it was seen that hydration of agar in calcium hydroxide exceeds that in water at 0.0001 N of the hydroxide, and this effect is also produced at 0.00001 N. Increase of hydration beyond that of water by dilute solutions of hydroxides of calcium, potassium, rubidium, potassium, sodium, and lithium, and excess values for aniline and ammonium hydroxides were obtained.

It was also seen that the strongest of the bases, potassium, in the form of a hydroxide would increase the swelling of agar-albumin mixtures to a point beyond that taking place in water alone.

The next logical step was to test the effects of salts of the common metals on swelling of the biocolloidal components. Here again the interesting fact was found that as chlorides, sodium and potassium at 0.001 M caused greater hydration of agar than water, the swelling being greater in the potassium. At 0.0001 M, sodium, potassium, magnesium, and calcium chlorides caused greater swelling than in water, the maximum swelling being in sodium, the next in potassium, and the least in calcium.

When chlorides of sodium and potassium were tested in series as shown in tables 1 and 2, it was found that pentosan-albumin mixtures showed increased hydration in the potassium chloride only as indicated in the tables.

TABLE 1. *Hydration of mixtures of agar 3 parts, gelatine 2 parts at 14° C. Plates 0.18 mm. in thickness; swelling of sections given in thickness and volume*

	0.01 M		0.001 M		0.0001 M.	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
HCL.....	550	600	930	1,025	1,430	1,575
KCl.....	1,900	2,015	2,270	2,530	2,440	2,640
CaCl.....	920	960	1,220	1,345	2,030	2,268
Water.....					2,200	2,330

⁴ MacDougal, D. T., and Spoehr, H. A. The components and colloidal behavior of plant protoplasm. Proc. Amer. Phil. Soc. 59: 154. 1920.

TABLE 2. *Hydration of mixtures of gelatine 3 parts, agar 2 parts at 14° C. Plates 0.18 to 0.19 mm. in thickness; swellings given in thickness and volume*

	0.01 M		0.001 M		0.0001 M	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
HCl.....	1,200	1,320	650	690	860	920
KCl.....	800	880	900	1,010	1,620	1,850
CaCl ₂	710	740	870	940	1,300	1,430
Water.....					1,275	1,420

The agar-gelatine mixtures are seen to have a higher capacity than the gelatine-agar, and also to react by high hydrations in greater concentration of potassium.

Up to this point colloids including only two of the supposed main elements in protoplasm have been used. An agar-gelatine mixture was now made to which was added a thousandth part of a soap which is probably nearly all sodium stearate. The results of the hydration swellings are given in table 3.

TABLE 3. *Hydration of plates of agar 3, gelatine 2, Ivory soap 0.005 g. at 15° C. Swellings given in thickness and volume*

	0.01 M		0.0002 M		0.0001 M	
	Th.	Vol.	Th.	Vol.	Th.	Vol.
NaCl.....	890	1,020			2,330	2,600
Balanced solution (Na 50 : Ca 1).....	810	842			2,460	2,660
CaCl ₂	850	920	1,200	1,374	2,600	2,940
KCl.....	970	1,050			2,750	2,950
HCl.....	1,280	1,460	1,130	1,270	1,200	1,250
Water.....					1,500	1,700

This biocolloid, representing more nearly the colloidal constitution of living matter, was seen to have higher hydration capacity in all salt solutions, and to have such capacity lessened in even the very dilute acid. A similar preparation in which the soap was pure potassium oleate gave results less marked as to the action of the salts, but the increase in the balanced solution was proportionately much greater, and the retarding effect in acids much greater. Ample justification exists, therefore, for a correction of the earlier statement as to the effect of salts of the common metals on biocolloids which have been found to offer many profitable analogies to living material.

The correction implies that we may confidently look to these salts as accelerators of hydration and growth, or as increasing the water deficit of living matter.

Lastly we come to the amino-acids and to water-soluble vitamines. I have previously pointed out in many papers that the commoner amino-acids, glycocoll, alanine, phenylalanine, asparagine, and histidine, which

have been proved to promote growth, also accelerate hydration in biocolloids.

As an additional step in this work, the effect of the water-soluble B yeast vitamine on biocolloids and living and dead cell-masses was measured. Solutions of this material at one tenth percent, having an acidity of PH = 5.25 as determined by the colorimeter method, were used, and measurements were taken by the auxograph. Taking the swelling in water as 100, the values obtained in the vitamine were as below:

Potato tubers, young, sections.....	75
Potato tubers, large, sections.....	230
Squash fruits, young, sections of pulp.....	110
Squash fruits, mature, sections of pulp.....	115
Orange seedlings, root tips, living.....	150
Orange seedlings, root tips, dried.....	120
Corn root tips, small, living.....	88
Corn root tips, large, living.....	78
Corn root tips, large, dried.....	180
Strawberry root tips, living.....	133
Sunflower stems, sections of pith, mature, living.....	150
Opuntia, sections of young joints.....	94
Opuntia, sections of mature joints.....	170
Opuntia, dried slices.....	90
Agar.....	140
Agar and soap.....	132
Agar and potassium oleate.....	80
Agar and phenylalanine.....	95
Agar 3, gelatine 2, and "salts".....	130
Agar 3, gelatine 2, soap.....	92
Agar 3, gelatine 2.....	136
Agar 3, gelatine 3.....	135
Agar 2, gelatine 3.....	143
Agar 2, gelatine 3, and "salts".....	130
Gelatine.....	163
Gelatine and soap.....	92

The vitamine is seen to increase the hydration swelling = water capacity or water deficit of agar, agar-soap, agar-gelatine, agar-gelatine-salts, and gelatine, but to lessen hydration in some biocolloids containing soaps which would be sensitive to the free hydrogen ions of the vitamine solution. Parallel action in living and dead cells is implied, although it is to be noted that such cells may already include a certain amount of their characteristic vitamines and that the added vitamine could exert no additional effect except that of reducing hydration capacity.⁵

SUMMARY

1. The theory as to the constitution of living matter by which plant protoplasm is taken to be a pentosan-albumin-soap colloidal mixture is

⁵ MacDougal, D. T. The effects of yeast vitamine water-soluble B on plant cell-masses and on biocolloids. Proc. Soc. Exper. Biol. and Med. 18 : 85. 1920.

briefly restated. Its adequacy at the present stage of experimentation is confirmed.

2. The metals which form the bases of nutrient salts, as hydroxides and as chlorides and nitrates, are found to increase the hydration capacity, or the water deficit of the principal components of biocolloids, and of biocolloids of certain composition.

3. Biocolloids containing soaps show a high degree of sensitiveness to hydrogen ions, or acidity. Such biocolloids show marked action in balanced solutions of sodium and calcium as shown by data too detailed to be given in this paper.

4. Yeast vitamine water-soluble B, in a solution slightly acid, increases the swelling, hydration, or water deficit in some living and dead plant cell masses, and lessens it in others. Similar diverse action on biocolloids was found.

5. All of the substances tested which are known to facilitate growth in plants are found to increase hydration capacity or water deficit in some of the test objects. The list includes chlorides and nitrates of sodium, potassium, magnesium, and calcium in various concentrations between 0.001 N and 0.0001 N, glycoll, alanine, phenylalanine, histidine, and water-soluble B yeast vitamine. Hydroxides of sodium, potassium, lithium, rubidium, calcium, ammonium, and aniline also increase hydration values in some of the components of living matter.